Epidermal Growth Factor Receptor Mutations in Small Cell Lung Cancer

A Brief Report

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Introduction: Knowledge about the current status of the epidermal growth factor receptor (*EGFR*) has resulted in an improvement in the treatment of non-small cell lung cancer. In contrast, small cell lung cancer (SCLC) continues to frustrate clinicians with its tendency toward early metastasis and chemotherapy resistance. Recent studies have reported the *EGFR* mutation and its response to gefitinib treatment in SCLC. We would like to share our experience of *EGFR* studies on SCLC patients.

Methods: Between 2004 and 2009, we prospectively collected 76 specimens from patients with SCLC at the National Taiwan University Hospital, Taiwan. These specimens included 10 computed tomography-guided biopsy specimens, 17 echo-guided aspiration specimens, 37 echo-guided biopsy specimens, 1 surgical lobectomy specimen, and 11 malignant pleural effusion specimens. Molecular genetic analysis of the specimens was conducted to detect the *EGFR* mutation.

Results: Among the 76 SCLC specimens we examined, 2 (2.6%) tested positive for the *EGFR* mutation and both were deletions in exon 19. One patient was administered gefitinib after several lines of chemotherapy but showed no treatment response. To date, only 11 *EGFR* mutant-positive SCLC patients, including our 2 patients, have been reported. Most of these patients were never smokers. The SCLC harboring *EGFR* mutation were more likely to be combined with adenocarcinoma compared with the whole SCLC population.

Conclusions: The *EGFR* mutation is rare in SCLC patients. Despite the presence of the *EGFR* mutation, gefitinib may not be effective in treating SCLC patients.

Key Words: Epidermal growth factor receptor mutation, Gefitinib, Small cell lung cancer.

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Small cell lung cancer (SCLC) accounts for 15% of all lung cancer cases. Because of its features, such as fast growth, early metastasis, and easy development of chemotherapy resistance, the overall 2-year survival rate in the extensive stage is merely 4.6%, whereas the overall 5-year survival rate in the limited stage is 10%.[1] Its molecular pathogenesis, although not thoroughly understood, was linked to autocrine-related cell growth, proto-oncogene activation, and tumor suppressor gene inactivation. Targeted treatments have been designed, but responses have been rather disappointing. Since 2004, epidermal growth factor receptor (*EGFR*) mutations in non-small cell lung cancer, such as deletions in exon 19 or L858R, were associated with a dramatic response to EGFR tyrosine kinase inhibitor (TKI) and improved survival.[2] However, another study failed to demonstrate antitumor activity of gefitinib on SCLC in both preclinical studies and a subsequent clinical trial.[3,4] In a phase II study, 2 of 20 SCLC patients reported stable disease with gefitinib treatment for more than 90 days, but *EGFR* status was not evaluated in the study.[4] For further treatment options, detection of *EGFR* mutation in unusual SCLC cases, such as cases of female nonsmokers with peripheral nodular lesions or concomitant adenocarcinoma, has been suggested.[5] Recent case reports and studies have focused on SCLC patients with the *EGFR* mutation, and some have described treatment responsiveness after EGFR TKI use.[6–8] To accumulate more evidence on potential novel treatment of SCLC, we reported our study results of *EGFR* mutation on patients of SCLC and reviewed relevant literature about *EGFR* mutation in SCLC.

PATIENTS AND METHODS

Between July 2004 and June 2009, 76 SCLC specimens were prospectively collected at the National Taiwan University Hospital, Taiwan. These specimens included 10 computed tomography (CT)-guided biopsy specimens, 17 echo-guided aspiration specimens, 37 echo-guided biopsy specimens, 1 surgical lobectomy specimen, and 11 malignant pleural effusion specimens. Tissues were collected at the time of first diagnosis after receipt of documented informed consent, and each sample was immediately immersed in RNA*later* (Qiagen, Valencia, CA). Patients' clinical data including demo-

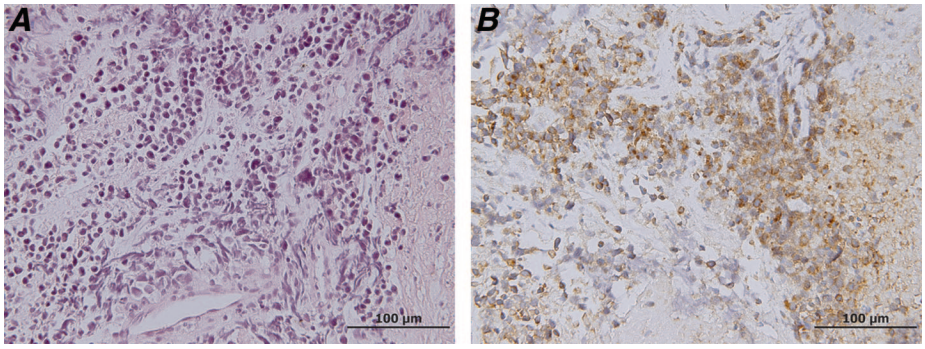

FIGURE 1. The pathology of a male smoker with epidermal growth factor receptor mutation-positive small cell lung cancer (SCLC). *A*, Tissue from an echo-guided lung biopsy. Under hematoxylin and eosin staining, SCLC showed characteristic small round oval cells with scanty cytoplasm and fine granular nuclear chromatin. Marked necrotic components and a crushed effect were noted. *B*, Strong positive immunochemical staining with synaptophysin.

graphic information, clinical staging, and smoking status were recorded. This study was approved by the Institutional Review Board of the National Taiwan University Hospital.

The *EGFR* mutation was assessed with reverse transcription polymerase chain reaction (RT-PCR) and direct sequencing. Exons 18 to 21, which are the four exons coded for the tyrosine kinase domain of the *EGFR* gene, were amplified with a forward primer (5′-AGC-TTG-TGG-AGC-CTC-TTA-CAC-C-3′) and reverse primer (5′-GGA-TCG-GCC-TCT-TCA-TGC-3′). Both the forward and reverse sequences were analyzed by basic local alignment search tool for cDNA of the *EGFR* gene (accession number NM005228), and the chromatograms were examined manually. Presence of the *EGFR* mutation was confirmed by subsequent independent RT-PCR and sequencing reactions. An *EGFR* mutation-positive result was recorded only if the mutation was identified in both rounds.

RESULTS

Of the 76 examined SCLC specimens, 2 were found to be *EGFR* mutation positive. The *EGFR* mutation rate in SCLC was 2.6% in our cohort. Three patients were diagnosed with lung cancer that had combined small and non-small cell pathology but showed no *EGFR* mutation. The median age of the *EGFR* wild-type patients was 71 years; among the patients, 64 (86%) were males, 63 (84%) were smokers, and 56 (75%) were in the extensive stage. Of the two patients with *EGFR*-mutated tumors, one was a male smoker and the other was a female never smoker. The diseases of both patients were at the limited stage. The mutations included two deletions in exon 19: del 2237–2255 insT (del E746-S752 insV) in one patient and del 2235–2249 (del E746-A750) in the other.

Case 1

The first patient was a 63-year-old male heavy smoker who reported a chronic cough with blood-tinged sputum for over 1 month and body weight loss of 7 kg over 2 months. Chest radiography showed a left hilar tumor, and chest CT revealed a mass in the left lower lobe with diffused mediastinum lymphadenopathy. He received echo-guided aspiration and biopsy. Microscopy revealed tumor nests of SCLC presenting with a marked necrotic component. Immunohistochemical stains revealed diffuse positivity of cytokeratin and synaptophysin (Figure 1). The initial staging was limited stage. Because the patient refused radiotherapy, chemotherapy was administered as first-line treatment. He received two courses of etoposide and cisplatin and was then shifted to topotecan because of an adverse reaction. Four courses of topotecan were administered, and a partial response was obtained. Subsequent radiotherapy of the residual tumor was performed. Unfortunately, diffuse liver and peritoneal metastases developed 2 months later, and the patient died 10 months after diagnosis.

Case 2

The second patient was a 54-year-old female never smoker who complained of chest tightness with right axillary radiation for several months. Chest plain radiography revealed a tumor in the upper right lung. Subsequent chest CT revealed a right hilar mass and a peripheral tumor in the right upper lobe. Echo-guided biopsy revealed a soft white specimen that contained hyperchromatic tumor cells that displayed marked necrosis under microscopy. SCLC was confirmed by immunoreactivity to cytokeratin, synaptophysin, neuron-specific enolase, and thyroid transcription factor-1 protein. The patient was diagnosed with limited-stage SCLC. Treatment was initiated with concurrent chemoradiation therapy with etoposide and cisplatin, and a partial response was obtained. Seven months after diagnosis, brain metastasis developed, for which whole-brain radiotherapy and two courses of topotecan were administered. Four months later, the patient developed liver and renal metastasis. Treatment was changed to doxorubicin at that time; however, bone metastases appeared 1 month later. Despite palliative radiotherapy, the patient's condition continued to deteriorate. Gefitinib was prescribed on the basis of the patient's *EGFR* mutation status; however, the disease continued to progress. Treatment was changed back to oral etoposide 85 days later, and the patient died 17 months after diagnosis.

DISCUSSION

We prospectively collected SCLC tissues and used RNA to perform RT-PCR and direct sequencing of *EGFR* exons 18 to 21. In this study, we found that the *EGFR* mutation rate was 2.6% and confirmed the findings of another report that stated that *EGFR* mutation was quite rare in SCLC.[8]

Between 2005 and 2006, two cases of *EGFR* mutation-positive SCLC were reported, and partial responsiveness to

TABLE 1. Comparison of SCLC Patients with *EGFR* Mutation

Patients	Author	Age/ Sex	Smoking Status	Diagnosis	Specimen	EGFR Mutation	Treatment/ Response	Tumor Location	Remarks
1	Araki et al.[9] and Okamoto et al.[7]	72/F	Never smoker	SCLC	Lung biopsy	Exon 19 del (delE746-A750)	Gefitinib[a]/partial response	Central	The patient was initially diagnosed adenocarcinoma and treated with gefitinib. SCLC was noted 3 d after gefitinib use. Patient died because of cerebral hemorrhage 5 mo later.
2	Zakowski et al.[6]	45/F	Never smoker	SCLC	Lung biopsy	Exon 19 del (delL747-P753insQ)	Gefitinib[a]/ progressed disease	No data	The patient was diagnosed adenocarcinoma and treated with erlotinib with partial response for 18 mo before diagnosis of SCLC.
3	Morinaga et al.[10]	46/F	Never smoker	SCLC	Lung biopsy	Exon 19 del (delE746-A750)	—	Peripheral	The patient was diagnosed adenocarcinoma with disease stabilization to gefitinib for 10 mo before diagnosis of SCLC.
4	Fukui et al.[5]	62/F	Never smoker	Combined SCLC/ adenocarcinoma	Lung resection	Exon 21 (L858R) both pathology	—	Peripheral	—
5	Tatematsu et al.[8]	36/F	Never smoker	Combined SCLC/ adenocarcinoma	Lung resection	Exon 21 (L858R)	—	No data	The patient was initially diagnosed adenocarcinoma with partial response to gefitinib for unspecified duration.
6		81/M	Smoker	SCLC	Lung biopsy	Exon 18 (G719A)	Gefitinib[a]/partial response	No data	—
7		69/M	Smoker	Combined SCLC/ adenocarcinoma	Lung biopsy	Exon 21 (L858R)	—	No data	—
8		89/F	Smoker	SCLC	Lung biopsy	Exon 21 (L858R)	—	No data	—
9		65/M	Smoker	Combined SCLC/ adenocarcinoma	Lung resection	Exon 19 del	—	No data	—
10	This study	63/M	Smoker	SCLC	Lung biopsy	Exon19 del (del E746-S752 insV)	—	Central	—
11		54/F	Never smoker	SCLC	Lung biopsy	Exon 19 del (del E746-A750)	Gefitinib[a]/progressed disease	Peripheral	—

[a] Dosage of gefitinib: 250 mg once a day.
EGFR, epidermal growth factor receptor; F, female; M, male; SCLC, small cell lung cancer; del, deletion.

gefitinib was noted in one patient.[6,7,9] Araki et al. and Okamoto et al. reported the case of a 72-year-old female never smoker who was initially diagnosed with adenocarcinoma by sputum cytology. Gefitinib treatment was initiated on the patient's request. However, a subsequent bronchoscopic biopsy conducted 3 days later yielded a diagnosis of SCLC. Direct sequencing showed an *EGFR* mutation with an exon 19 deletion (delE746-A750). Gefitinib treatment showed amazing responsiveness both in the primary tumor and in the liver metastasis. The patient died 5 months later because of cerebral hemorrhage.[7,9] Zakowski et al. reported the case of a 45-year-old female never smoker who was diagnosed with lung adenocarcinoma by bronchoscopic biopsy. Her initial *EGFR* status was unknown, but the tumor showed partial regression after erlotinib treatment. Eighteen months later, a brain metastasis developed, and the tumor showed no responsiveness to gefitinib. A second lung biopsy revealed SCLC with *EGFR* exon 19 deletion. Autopsy showed multiple-organ metastases of SCLC without any adenocarcinoma.[6] In 2007, Morinaga et al.[10] reported the case of a 46-year-old Japanese female never smoker who was diagnosed with sequential adenocarcinoma and SCLC with the same *EGFR* mutation. Fukui et al.[5] focused on the relationship between combined SCLC and *EGFR* mutation and reported the case of a 62-year-old female never smoker who had the L858R mutation at both the SCLC and the adenocarcinoma parts. In 2008, a study on 122 SCLC patients described 5 patients (4%) who harbored the *EGFR* mutation.[8]

To date, nine *EGFR* mutation-positive SCLC cases have been reported in six published studies.[5–10] In this study, we encountered two new SCLC patients with the *EGFR* mutation. The mutations of all reported patients include six deletions in exon 19, four in L858R, and one in G719A. The most enchanting question is what are the clinical characteristics of SCLC patients with *EGFR* mutations? To answer this question, we have summarized all 11 cases in Table 1.

These 11 *EGFR* mutant-positive SCLC patients had distinct clinicopathologic features of the epidemiologic data of the SCLC population. The median age was 62 years. The gender distribution showed female predominance (63%).

Smokers comprised only 45% of the group, which was much lower than previously reported data (74.1–97.5%).[1] Radiologically, three patients showed peripheral lesions, two showed central lesions, and the other six were not described in the original publication. Four patients (patients 4, 5, 7, and 9 in Table 1) had combined SCLC and adenocarcinoma. Histologically, the incidence of the combined pathology was higher compared with previous SCLC data (36% versus 1.2%–28%[11,12]). Four patients (patients 1, 2, 3, and 5 in Table 1) were diagnosed with adenocarcinoma before being diagnosed with SCLC. In summary, *EGFR* mutant-positive SCLC would probably be more common in never smokers who had SCLC combined with adenocarcinoma.

The treatment response of EGFR TKI may be better clarified when we distinguish the 11 patients into 2 groups, those with *EGFR* mutant-positive SCLC at the time of diagnosis (patients 1, 4, 6, 7, 8, 9, 10, and 11 in Table 1) and those with *EGFR* mutant-positive SCLC after gefitinib treatment (patients 2, 3, and 5 in Table 1). Three of the eight mutant-positive patients who confirmed at the time of diagnosis (patients 1, 6, and 11) received gefitinib treatment, which resulted in two partial regressions (patient 1 with del E746-A750 and patient 6 with G719A) and one disease progression (patient 11 with del E746-A750). Among the three patients with confirmed *EGFR* mutation and prior gefitinib treatment, only one patient (patient 2 with del L747-P753insQ) received gefitinib treatment but experienced disease progression.

In other studies, *EGFR* mutation was a significant predictor of survival in patients with adenocarcinoma treated with gefitinib.[2] This result is different from that observed in SCLC. This response inconsistency may be due to the *EGFR* mutation playing only part of the role in SCLC development or may be related to limited case numbers. If we were able to obtain repeated tissue proof at the time of recurrence, we might have further evidence to explain the suboptimal treatment.

Judging by the 11 cases reported so far, the incidence of *EGFR* mutation in SCLC is low and may be related to the distinct clinicopathologic presentation of SCLC, particularly among never smokers and those with mixed histology. Testing for *EGFR* mutations in SCLC tumors is generally not advised; however, it may be considered in cases where the patient is a never smoker or has mixed histology. Gefitinib may not be effective for treating SCLC patients despite the patients having a positive *EGFR* mutation status.

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